KARYOTYPES OF *MUHLENBERGIA RIGIDA* (POACEAE: CHLORIDOIDEAE) FROM NORTH CENTRAL MEXICO

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ABSTRACT

Karyotypes from two morphotypes in 30 populations of *Muhlenbergia rigida* (Kunth) Trin. from locations in six states in north central Mexico were analyzed. The basic ploidy ($x = 10$), tetraploidy ($n = 40$) and disploidy ($n = 44$) reported previously for the species are confirmed, and triploidy ($n = 30$) is reported for the first time in 13 of the 30 populations studied. Analysis of karyotypes reveal a correlation among cytotypes and morphotypes of *M. rigida*. Triploid cytotypes correspond to the morphotype having open inflorescences whereas tetraploid and disploid plants correspond to the morphotype with dense inflorescences.

KEY WORDS: grasses, karyology, *Muhlenbergia rigida*, ploidy level

RESUMEN

Se presentan los cariotipos de dos morfotipos en 30 poblaciones de *Muhlenbergia rigida* (Kunth) Trin. provenientes de localidades en seis estados del norte-centro de México. Se confirma el número básico ($x = 10$), tetraploidía ($n = 40$) y disploidía ($n = 44$) reportados anteriormente para la especie, y se registra por primera vez triploidía ($n = 30$), en 13 de las 30 poblaciones estudiadas. El análisis de los cariotipos revela una correlación entre los citotipos y los morfotipos de *M. rigida*, con los citotipos triploides relacionados con el morfotipo de inflorescencia abierta, mientras que los tetraploides y disploides corresponden al morfotipo de inflorescencia densa.

PALABRAS CLAVE: cariología, gramíneas, *Muhlenbergia rigida*, nivel de ploidía

INTRODUCTION

Muhlenbergia is one of the largest genera of the Poaceae family, with approximately 160 species worldwide. Of those, 150 are distributed mainly in the temperate forests and grasslands located on the American continent; only six species are endemic to South Asia (Clayton & Renvoize 1986; Peterson 2000; Peterson & Annable 1991; Peterson & Ortíz-Díaz 1998; Peterson and Herrera 2001; Watson & Dallwitz 1992). There are around 133 species of *Muhlenbergia* native to the Southern United States of America and Northern Mexico, some of them even reaching Central America (38) and South America (25). The majority of the species (120) are located in Mexico (Beetle 1986; Espejo-Serna et al. 2000; Espejo Serna 2012; Herrera Arrieta 1987, 1998; Herrera Arrieta & de la Cerda Lemus 1995; Herrera Arrieta & Peterson 1992; Peterson et al. 2001; Peterson 2003; Peterson & Valdés-Reyna 1999). *Muhlenbergia* is a genus with great morphological variation, especially within vegetative characters, ranging from very small plants (5 to 40 cm tall) in *M. minutissima* (Steud.) Swallen to very large ones (150–300 cm tall) in *M. robusta* (E. Fourn.) Hitchc. Furthermore, *M. rigida* (Kunth) Trin. has populations

that differ in plant habit as well as in the shape and size of their inflorescence, which can be compact (dense) or loose (open), large or small. This creates taxonomic confusion and problems with the specific delimitation.

Few chromosome studies have been carried out on the genus *Muhlenbergia*. Soderstrom (1967) stated that Avdulov (1931) was the first who reported cytological information as a basis for a new classification system of Poaceae. Although Sodertstrom (op. cit.) did not carry out cytological studies, he does mention the results from several studies which reported a base chromosome number for *Muhlenbergia* of $x = 9$, 10, or 21. These studies included the $2n$ chromosome number for the species of sect. *Epicampes*. Peterson (1988) studied the chromosome number of 25 annual *Muhlenbergia* species, reporting for the first time the chromosome number of nine of them. Noteworthy among these are *Muhlenbergia biloba* Hitchc. and *M. shepherdii* (Vasey) Swallen since they are $n = 8$. Herrera (1995) published the chromosome number of three species of the *M. montana* (Nutt.) Hitchc. complex [(*M. montana, M. quadridentata* (Kunth) Trin., and *M. virescens* (Kunth) Trin.)], recording for the first time the chromosome number of *M. quadridentata*, the disploid chromosome number of *M. virescens* and confirming the tetraploid condition of *M. montana*. Gould (1966) carried out karyotyping of 60 genera and 149 species of Mexican grasses, eight of which belong to *Muhlenbergia*. However, *M. rigida* was not included in Gould's study.

Muhlenbergia rigida is a grass with widespread distribution in the SW United States and ranges south to Central and South America. In Mexico it is found from Baja California to Chiapas. The grass can be found growing in pine-oak forests and grasslands, sporadically at the edge of roads, and at elevations between 1280–2550 m (Herrera Arrieta & Peterson 2007).

This capacity to grow in such extensive areas with diverse habitats translates into a great morphological diversity within the species (rigida). This diversity of traits is reflected mainly in the shape and size of leaves and inflorescences, with variation among the type of inflorescence (compact or loose) being particularly notorious. However, there is no correlation between morphotypes and habitats or geographical distribution as frequently both morphotypes can be found growing in the same location. On the basis of the great morphological variation of *M. rigida* and the complementary information that is generated in an inter-population cytogenetic study, the aim of the present study is to compare the karyotype of two morphotypes in 30 populations of *M. rigida* located in north central Mexico. This comparison will reveal any relationship between the karyotype and the morphological differences within the species as well as other relevant variations that allow the distinction of groups among the studied populations, or otherwise distinguish between the two morphotypes.

MATERIALS AND METHODS

Plant material

Sample material was collected from 27 locations (Fig. 1) and 30 populations of *Muhlenbergia rigida*, following its distribution in the states of Chihuahua, Durango, Zacatecas, Aguascalientes, Guanajuato, and Jalisco, Mexico (Table 1). Live plants were collected (bunches) from natural populations and cultivated under greenhouse conditions in order to collect the roots required for this study. Table 1 lists the voucher, disploid number, and localities (including geographical coordinates and elevation) in which the studied plants were collected.

Karyotyping

Karyotypes were studied using roots obtained from adult plants cultivated in the greenhouse. When roots reached approximately 1–1.5 cm in length, they were subjected to a treatment with an 8-hidroxiquinolein (2 mM) solution in darkness for 3 hours at room temperature. Later they were fixed with ethanol/acetic acid (3:1) for 12 hours at 4°C, followed by hydrolysis with three passes using distilled water at 30-minute intervals, and then acid hydrolysis using 0.1N HCl, and citrates buffer for 30 minutes, then a final digestion using a 4% cellulase enzyme mixture "Onozuka R-10 (Serva)" and 1% pectinase Y-23 (Seishin Pharmaceutical) for 1 hour at 37°C. After a quick wash using distilled water, roots were kept for one minute in 45% acetic acid followed by the corresponding squashing. Cover slides were removed after the samples were kept at -84°C. Preparations were left to dry for 1 day at ambient temperature. Chromosomes were stained using the acetocarmine reagent. Counting, analysis, and interpretation of the chromosomes (in 7–10 metaphase slides) was carried out using an

Fig. 1. Map showing the collection localities of *Muhlenbergia rigida*.

Axioskop Zeiss microscope fitted with digital camera. The MicroMeasure software http://www.colostate.edu/Depts/Biology/MicroMeasure) was used for measuring the length of each chromosome.

The ratio of the chromosome arms (r) (long arm: short arm) was also calculated, while the nomenclature used for describing chromosome morphology was that proposed by Levan et al. (1964): m = metacentric (r: 1.00–1.69), sm = submetacentric (r = 1.70–2.99), and st = subtelocentric (r = 3.00–6.99). Stebbins' standardization (1938) was used for classifying chromosomes according to their length: <2 µm, small; 2–4.9 µm, medium-small; 5–9 µm, medium-large; >9 µm, large.

In order to carry out the quantitative characterization of the karyotypes the following parameters were

Table 1. *Muhlenbergia rigida* populations with karyotype studies.

Population	2n	Locality
O. Rosales 3970	40	5.3 km Al NW de La Hacienda La Pila, Dgo., 24°07'44.8"N y 104°18'29.3"W, 1884 m
O. Rosales 3976	40	Rancho Los Yugos, San José de Gracia, Ags., 22°00'00.2"N y 102°34'38.8"W, 2369 m
O. Rosales 3982	40	3.3 km al W de La Parrilla, Dgo., 23°43'39.6"N y 104°28'22.92.2ºW, 2260 m
O. Rosales 3990	30	2.7 km. del entronque carretera federal libre 40 hacia la carretera federal de cuota 40, Durango, Dgo. 23°56'55.7"N y 104°56'15.3"W, 2415 m
O. Rosales 3993	30	Puente Río Chico, carretera de cuota Durango-Mazatlán, Durango, Dgo.23°58'38.0"N y 104°53'32.5"W, 2256 m
O. Rosales 4016	44	1 km del entronque Jiménez del Teul hacia Molina de Agua Zarca, Jiménez del Teul, Zac. 23°21'00.01"N y 103°51'10.2"W, 2271 m
O. Rosales 4028	30	Km 168 carretera Sombrerete-Durango, Sombrerete, Zac. 23°39'52.7"N y 103°40'23.1"W, 2425 m
O. Rosales 4029	30	Sierra de Órganos, Sombrerete, Zac., 23°44'38.7"N y 103°48'22.3"W, 2296 m
O. Rosales 4030	40	Sierra de Órganos, Sombrerete, Zac. 23°44'39.6"N y 103°48'02.0"W, 2329 m
O. Rosales 4053	30	Sierra del Registro por carretera federal 23, Durango, Dgo. 23°45'51.6"N y 104°25'29"W, 2014 m
O. Rosales 4098	44	Parte alta de la Cañada el Cajón, Santa María, El Oro, Durango, 25°24'42.8"N y 104°57'28.4"W, 1914 m
O. Rosales 4124	40	Entronque Otinapa-Autopista Durango-Mazatlán, Durango, Dgo. 23°58'35.0"N y 104°56'59.7"W, 2446 m
O. Rosales 4140	40	Km 25 Autopista Durango-Torreón, Durango, Dgo. 24°11'12.0"N y104°29'31.7"W, 1864 m
O. Rosales 4146	30	Por la carretera 105, rumbo a la presa Bayacora, Durango, Dgo., 34°54'44.1"N y 104°44'46.9"W, 2185 m
O. Rosales 4158	30	Cerro de Los Gallos, Aguascalientes, Ags. 21°40'03.6"N y 102°13'15.8"W, 2191 m
O. Rosales 4159	40	Cerro de Los Gallos, Aguascalientes, Ags. 21°40'03.6"N y 102°13'15.8"W, 2191 m
O. Rosales 4162	40	Entronque a Milpillas, Jesús María, Ags. 21°55'28.6"N y 102°33'57.7"W, 2186 m
O. Rosales 4166	30	Cerro El Roble, Jesús María, Ags., 21°47'30.7"N y 102°31'26.3"W, 2019 m
O. Rosales 4170	40	6 km antes de la caseta de cobro por la autopista Aguascalientes-Zacatecas, Guadalupe, Zac., 22°39'19.6"N y 102°26'45.5"W, 2305 m
O. Rosales 4177	44	1.5 km al SE de Canutillo, Sombrerete, Zac. 23°35'26.0"N y 103°46'22.0"W, 2119 m
O. Rosales 4187	30	Carretera 24 rumbo hacia Guadalupe y Calvo, Chih. 26°42'27.2"N y 106°04'15.9" W, 2296 m
O. Rosales 4202	30	Carretera Chihuahua-Cuauhtémoc, Chih. 28°28'04.3" y 106°11'50.7"W, 1711 m
O. Rosales 4203	30	Al sur la ciudad de Guerrero, Guerrero, Chih. 28°30'15.8"N y 107°29'00.3"W, 2045 m
O. Rosales 4210	30	Carretera 16 Yecoma-Cuauhtémoc, Chih. 28°24'09.4"N y 107°34'57.0"W, 2277 m
O. Rosales 4215	40	Carretera 16 Yecoma-Cuauhtémoc, al norte de Temochi, Chih., 28°21'20.7"N y107°49'26.0"W, 2075 m
O. Rosales 4219	40	Limites Cd. Cuauhtémoc-Bachiniva, Chih. 28°48'34.6"N y 107°11'59"W, 2260 m
O. Rosales 4227	40	Entronque al Soyatal por carretera Villa Hidalgo- Teocaltiche, Jal. 21°34'55.6"N y 102°34'26.4"W, 1910 m
O. Rosales 4234	30	Carretera Nochistlan-Yahualica, Yahualica, Jal. 21°14'46.3"N y 102°48'53.4"W, 1798 m
O. Rosales 4235	44	Km 25 carretera Yahualica-Jalostotitlan, entronque a Mezcala de los Romero, Jal. 20°57'44.0"N y 102°48'57.8"W, 1820 m
O. Rosales 4252	30	Sierra de Lobos por carretera Ocampo-León, Gto. 21°13'24.7"N y 101°36'40.3"W, 2544 m

calculated: (1) total chromosome length (LTC); (2) mean chromosome length (LMC); (3) centromere index mean (short arm/total chromosome length x 100 [CI]); (4) intra-chromosomal asymmetry index (A1) = 1-[$\Sigma(b/B/n)$, where b and B are the measures of the short and long arm of each homologous chromosome pair respectively and n is the total number of homologues; (5) inter-chromosomal asymmetry index (A2) = s/x, where s is the standard deviation and x is the mean chromosome length; (6) Paszko Index AI = CVCL x CVCI/100 where CVCL= (SCL/XCL) x 100 is the relative variation of chromosome length, CVCI = (SCI/XCI) x 100 is the relative variation of the centromere length, respectively, XCL is the mean chromosome length and XCI is the CI mean. Karyotype asymmetry was determined using Stebbins' categories (1971), A1 and A2 indices (Romero-Zarco 1986) and the AI index (Paszko 2006). The A1 index is a quantification of Stebbins' asymmetry categories. It ranges between 0 and 1, and these are low when chromosomes tend to be metacentric. Basic interpretation of AI values determines that the higher the value, the higher is the heterogeneity of chromosome length and/or the centromere index in a studied karyotype (García-Barriuso et al. 2010).

In the ideograms, homologue chromosome pairs were ordered according to their length in decreasing-size order. Four to seven metaphase cells were measured from various slides in order to obtain an average for construction of the ideograms. Measurements were compared using ANOVA. The TCL, CI, A1 and A2 indices as well as chromosome number were considered.

Clustering analysis of the karyotype data was carried out in order to examine karyotype similitude among populations. A data matrix of 30 OTUs (operational taxonomic units) and five variables was constructed. The following variables were used: LMC, CI, A1, A2 and chromosome number. The first four variables were used because they are not influenced by chromosome number. Nevertheless, ploidy level was also used since different characteristics of various populations of *Muhlenbergia rigida* morphotypes are being compared. The STATISTICA v.7.0 (StatSoft, 2004) software package was used to normalize the data matrix, calculate the average Euclidean distance, and generate an UPGMA dendrogram.

Also, in order to evaluate the contribution of each karyotype parameter to the population clustering, the entities were subject to a principal component analysis (PCA) based on the 30 OTU data matrix and the aforementioned five quantitative variables.

RESULTS

The chromosome counts obtained from radicular cells of *Muhlenbergia rigida* in this study are consistent with the numbers reported by Herrera and Peterson (2007) and Soderstrom (1967), both of which documented $2n$ = 40 and 44. This study recorded $2n$ = 30, 40, and 44; marking the first report of triploidy in this species. It is noteworthy to mention that the $2n$ = 40 and $2n$ = 44 counts were found in the compact panicle morphotype while the $2n$ = 30 count was present in the open panicle morphotype.

Our results support previous suggestions that the basic number of *Muhlenbergia* is x = 10, with $2n$ = 40 occurring in the majority of the species. Polyploidy is equally distributed in the morphotypes of *M. rigida* studied here: 43.33% of the plants are tetraploid ($2n$ = 4x = 40), 43.33% are triploid ($2n$ = 3x = 30) and only 13.33% are disploid, derived from tetraploid ($2n$ = 4x + 4).

We report for the first time the karyotype formula of 30 chromosomes in *M. rigida* populations as well as the ideograms representing the three ploidy levels found (Fig. 2)

As a whole, the karyotypes of the species analyzed were composed of metacentric (m) and submetacentric (sm) chromosomes, with the former being predominant. The formulae among triploid populations were 30m (six populations), 29m + 1sm (three populations), 27m + 3sm (two populations), and 25m + 5sm and 22m + 8sm in the remaining two populations.

Tetraploid populations were present in the following manner: 40m (five populations), 39m + 1sm (three populations), 38m + 2sm, 37m + 3sm, 35m + 5sm, 32m + 8sm, 34m + 6sm, 32m + 8sm in five populations. Disploid populations had the following karyotype formulas: 44m (two populations) and 30m + 14sm, 35m + 9sm for the remaining two populations (Table 2).

Chromosomes were small and medium-small in size (between 1.0 and 3.85 µm), according to Stebbins (1938). The mean chromosome length (LMC) ranged between 1.31 µm and 2.62 µm. The centromere index varied from 12.94 to 21.76.

In general the karyotypes were moderately symmetrical and fall into categories 1A and 1B of Stebbins (1971).

The UPGMA dendrogram constructed with karyotype similitudes (Fig. 3) shows three main groups divided into subgroups. The first group is composed of three subgroups with group I-1 being composed of populations 4098, 4177, and 4016. Group I-2 was formed by populations 4215, 4140, 4170, 4124, and 4053. Group I-3 was composed of populations 4227, 3982, 4159, 4219, 4030, 4162, and 3976; and was characterized by intermediate CI value (A_1 = 0.10 to 0.14). Group two contained only two populations, 4235 and 3970; with low CI (A1 = 0.15 to 0.19). The third group was composed of three subgroups with the following populations: subgroup III-1 by 4252, 4029, and 4028, with intermediate CI value (A1 = 0.12 to 0.16); subgroup III-2 by 4203, 4166, 4210, 4234, 4202, 4187, and 4158 also with intermediate CI value (A1 = 0.03 to 0.16); and subgroup III-3 by 3993, 4146, and 3990. It is important to note that this separation into groups and subgroups is intimately related to the morphological characteristics of the inflorescence and ploidy levels. The first group contains disploid and tetraploid populations, the second group has one disploid and one tetraploid population, while the third group is completely composed of triploid populations that have an open panicle inflorescence.

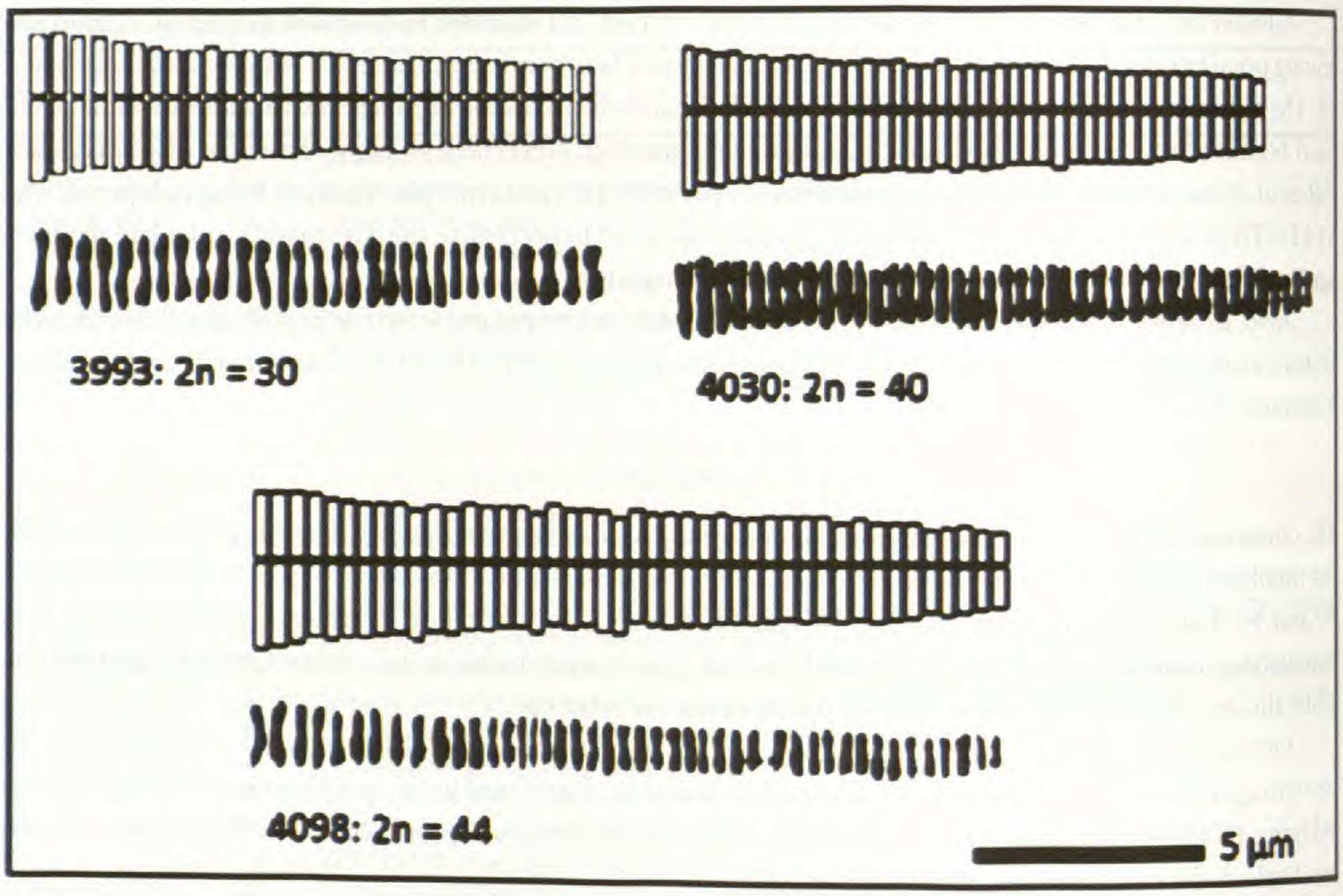

Fig. 2. Ideograms representing the three ploidy levels of *Muhlenbergia rigida* populations. Bar scale = 5 µm.

Measurements from triploid and tetraploid plants from the same locality were statistically compared resulting in significant differences ($P < 0.05$).

DISCUSSION

The results obtained from our study of *Muhlenbergia rigida* morphotypes partially confirms the previously reported chromosome numbers for this species by several authors.

Of the 30 populations examined, 13 were found to be triploid, this being the first report for triploidy in the species. Apart from the triploid cytotypes this study also found tetraploid and disploid plants, confirming the results of previous studies. The triploid and tetraploid cytotypes are present in high frequency (86%) while disploidy ($2n = 44$) had low frequency, being found in only four populations (Table 2).

This study presents ploidy levels found in *M. rigida* populations in north central Mexico. It also shows, for the first time, the karyotype and ideograms of the two *Muhlenbergia rigida* morphotypes (dense and loose panicle) as well as the three cytotypes within the species.

Polyploidy has played an important role in the evolution of many eukaryotes (Soltis et al. 1999), and the majority of angiosperms (approximately 70%) have shown polyploidy during their evolutionary process (Masterson 1994). It is highly probable that the polyploidy of *Muhlenbergia rigida* is of autopolyploidy in origin, since the cytotypes have very similarly sized chromosomes. General morphology of the studied plants was very similar (Table 2).

The results of this study reveal a detailed description of the chromosomal traits of two *Muhlenbergia rigida* morphotypes, allowing the classification of the karyotype of *M. rigida* as symmetrical. The chromosomes are small in size with an average length that does not surpass 2.87 mm, according to the classification of Stebbins (1971).

Taking into account the relative abundance of polyploids when compared to their disploid ancestors, Stebbins (1971) recognizes five maturity stages of polyploidy complexes: initial, young, mature, declining, and

TABLE 2. Populations of *Muhlenbergia rigida*: voucher number and morphotype (d = dense panicle, o = open panicle), chromosome number (*2n*), karyotype formula (KF), total chromosome length (TCL), long arm length (LCL), short arm length (SCL), mean chromosome length (LMC), centromere length (CI), intra-chromosome asymmetry index (A_1), inter-chromosome asymmetry index (A_2), Paszko asymmetry values (CV_{CI} x CV_{CL}), Paszko asymmetry index (AI), Stebbins asymmetry category(ST), SE standard error.

Populations	2n	KF	TCL±SE (µm)	LCL (µm)	SCL (µm)	LMC±SE (µm)	CI±SE	A1	A2	CVCI	CVCL	AI	ST
3970(d)	40	40m	61.29±0.97	0.84	0.69	1.53±0.02	19.63±0.07	0.98	0.15	7.77	15.35	1.19	1A
3976(d)	40	40 m	46.68±2.94	0.85	0.71	1.6±0.09	14.52±0.04	0.98	0.10	7.67	10.33	0.79	1A
3982(d)	40	40 m	45.94±0.71	0.83	0.70	1.53±0.02	15.06±0.07	0.98	0.10	6.42	10.02	0.64	1A
3990(o)	30	29m+1sm	72.95±4.88	1.37	1.03	2.40±0.17	13.01±0.07	0.97	0.19	10.69	18.82	2.02	1B
3993(o)	30	29m+1sm	72.01±5.56	1.27	0.98	2.40±0.19	13.95±0.06	0.97	0.21	10.41	20.62	2.12	1B
4016(d)	44	35m+5sm	59.52±7.94	1.14	0.85	1.98±0.12	14.86±0.05	0.98	0.13	9.69	12.68	1.23	1B
4028(o)	30	25m+5sm	39.50±1.83	0.74	0.58	1.32±0.06	14.48±0.04	0.97	0.17	10.15	16.80	1.71	1B
4029(o)	30	27m+3sm	44.64±1.15	0.83	0.65	1.49±0.04	14.62±0.06	0.97	0.17	11.03	17.42	1.92	1B
4030(d)	40	32m+8sm	55.59±2.60	1.05	0.81	1.85±0.08	14.44±0.07	0.98	0.14	10.03	13.81	1.39	1B
4053(d)	40	38m+2sm	70.56±3.09	1.38	0.98	2.35±0.10	13.86±0.04	0.98	0.14	10.24	13.8	1.41	1B
4098(d)	44	30m+14sm	59.58±1.65	1.12	0.87	1.99±0.05	14.15±0.06	0.98	0.12	9.32	11.96	1.12	1B
4124(d)	40	39m+1sm	63.32±4.26	1.06	0.91	2.11±0.14	14.18±0.09	0.98	0.16	11.15	16.36	1.84	1A
4140(d)	40	37m+3sm	55.79±4.71	1.06	0.80	1.86±0.16	13.85±0.06	0.98	0.14	10.81	13.84	1.50	1A
4146(o)	30	22m+8sm	64.55±4.37	1.22	0.90	2.12±0.14	12.94±0.07	0.98	0.15	12.42	15.22	1.89	1B
4158(o)	30	27m+3sm	47.26±2.11	0.89	0.69	1.58±0.07	13.43±0.06	0.97	0.19	9.89	18.53	1.83	1B
4159(d)	40	39m+1sm	53.07±6.29	0.99	0.78	1.77±0.21	14.56±0.04	0.98	0.11	9.51	11.14	1.06	1B
4162(d)	40	34m+6sm	49.66±0.91	0.92	0.74	1.66±0.03	14.58±0.04	0.98	0.11	8.20	11.21	0.91	1A
4166(o)	30	30m	52.74±2.15	1.06	0.85	1.76±0.08	13.37±0.05	0.97	0.16	9.09	16.46	1.49	1B
4170(d)	40	35m+5sm	56.50±3.76	1.06	0.82	1.88±0.12	14.20±0.04	0.98	0.11	10.94	10.69	1.16	1A
4177(d)	44	44m	56.36±2.23	0.95	0.80	1.75±0.07	14.99±0.05	0.98	0.12	7.96	11.79	0.94	1A
4187(o)	30	30m	48.48±2.33	0.98	0.72	1.62±0.08	13.55±0.05	0.97	0.16	8.78	16.11	1.42	1A
4202(o)	30	30m	48.76±3.04	0.90	0.73	1.62±0.10	13.51±0.05	0.97	0.15	8.33	14.74	1.22	1A
4203(o)	30	30m	55.51±2.01	1.03	0.82	1.85±0.07	13.29±0.05	0.97	0.15	8.75	14.95	1.31	1A
4210(o)	30	30m	52.74±1.98	0.97	0.78	1.76±0.07	13.41±0.05	0.97	0.17	1.53	3.11	0.05	1A
4215(d)	40	39m+1sm	78.46±5.11	1.45	1.16	2.62±0.17	14.57±0.07	0.98	0.13	10.84	12.59	1.36	1[a]
4219(d)	40	40m	56.70±2.74	1.05	0.84	1.89±0.09	14.52±0.06	0.98	0.13	10.65	12.76	1.36	1B
4227(d)	40	40m	61.74±2.18	1.14	0.92	2.06±0.07	14.98±0.09	0.98	0.14	7.34	13.81	1.01	1B
4234(o)	30	29m+1sm	39.23±2.52	0.72	0.59	1.31±0.08	13.47±0.04	0.97	0.16	8.95	15.62	1.40	1B
4235(d)	44	44m	64.99±4.91	1.18	0.98	2.17±0.16	21.76±0.02	0.98	0.12	8.49	11.95	1.01	1B
4252(o)	30	30m	60.15±1.93	1.11	0.90	2.01±0.06	14.65±0.02	0.97	0.16	8.62	16.09	1.38	1B

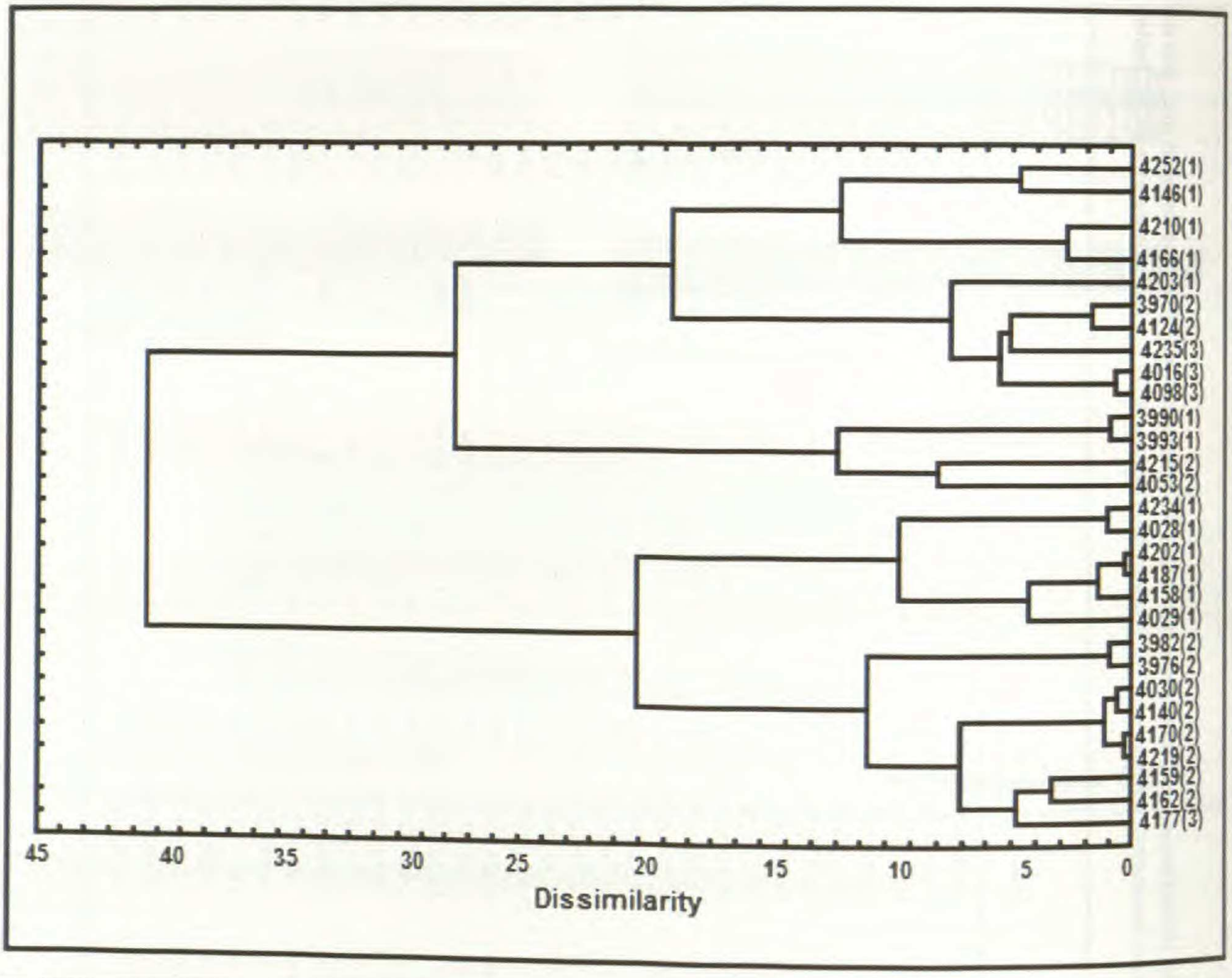

Fig. 3. Dendrogram showing the phenetic relationships among the *Muhlenbergia rigida* populations constructed using a karyotype similitude matrix with (UPGMA). (1 = triploid, 2 = tetraploid, 3 = disploid).

relictual. According to him, the evidence of distribution patterns indicates that the majority of polyploidy complexes that are currently mature originated in the Pliocene or Pleistocene. Our finding of 100% polyploidy frequency in the *Muhlenbergia rigida* populations indicates a high evolutionary maturity.

Chromosomes of studied populations can be classified from medium-small to medium-large based on Stebbins' terminology (1971). All populations have predominantly metacentric chromosomes and, to a lesser degree, submetacentric chromosomes. The A1 index fluctuated 0.1; for example, 50% of the populations had a 0.97 value while the other 50% had 0.98, which supports the predominance of metacentric chromosomes.

The *Muhlenbergia rigida* karyotype constitutes the first karyotypic description of the genus. Karyotype asymmetry was reflected in the various index values (A1, A2, AI, and Stebbins categorization). According to Stebbins' classification (1971), the karyotype asymmetry is type A1 and B1. This degree of asymmetry between populations is high, reflecting low specialization (Stebbins 1971).

The inter-chromosome asymmetry index A2 values varied from 0.10 to 0.21, indicating a similar chromosome length in most of the populations. The highest variation was observed in population 3993, where A2 was 0.21, while the lowest was observed in populations 3976 and 3982, where A2 was 0.10.

The Paszko's asymmetry (2006) calculated values (Table 2) show a low asymmetry, as the values range from 0.05 to 2.12. The most symmetrical karyotype was present in population 4210 which had a 0.05 AI value, while the most asymmetrical karyotypes were present in population 3990 (with an AI of 2.02) and population 3993 (with an AI of 2.12).

The agglomerative clustering analysis (UPGMA) revealed that the parameters which had greater influ-

ence in group and subgroup formation were the centromere index (CI) and inter-chromosome asymmetry (A2). The CI values had an interval of 12.94 to 21.76 while the inter-chromosome asymmetry index values (A2) range from 0.10 to 0.21, marking clear difference between morphotypes. Populations with the open panicle morphotype had the highest A2 values (0.15 to 0.21) while the closed panicle morphotype populations had lower A2 values (0.10 to 0.16).

The amount of metacentric chromosomes in the cytotypes studied, suggest that the karyotype of this species shows a tendency to be symmetrical, indicating a trend to become stable.

The ploidy levels of *Muhlenbergia rigida* are related to morphotypes. Plants that had compact inflorescence were tetraploid and disploid ($2n = 40$ and 44) while those with loose inflorescence were triploid ($2n = 30$). Therefore, cytological data provides a good complement to taxonomic studies. Knowledge on simple cytogenetic characteristics of a species such as chromosome number, behavior of the chromosomes during meiosis, the mode of reproduction of individuals and their fertility can contribute to a better understanding of the patterns of morphological variation and help to define taxonomic limits. The results obtained in this study confirm the differences between two morphotypes of *M. rigida*, which maintain their morphological and cytological features even when growing at the same location, and may represent two taxonomic entities. However, further evidence may be required to support their recognition at the species level.

The lack of cytogenetic information on species of *Muhlenbergia* makes the comparative study of karyotypes and their quantitative characteristics difficult, limiting deeper discussion on the possible participation of chromosome changes in the evolution of the genus, in its speciation processes, and the establishment of some type of genome specialization in relation to the habitat. It is expected that a thorough cytogenetic study (with banding, FISH, or GISH) could provide more elements to determine the evolutionary history of *M. rigida* morphotypes.

ACKNOWLEDGMENTS

We want to thank Instituto Politécnico Nacional (CIIDIR-DGO, SIP, and COFAA) for financial support, and the grants given to the following projects: SIP-20010344 and CONABIO-VO24 "Revisión de *Muhlenbergia* (Poaceae) de México," SIP-20031025 "Estudio de los pastos más comúnes de México," SIP-20110017 "Citología de gramíneas y ciperáceas de importancia económica del n-c de México," and SIP-20110681"Fitodiversidad y Ecosistemas de la Sierra Madre Occidental." Many thanks to CONACyT by the scholarship to the graduate student (CVU: 242097), first author of the present report. Finally we want to thank the two anonymous revisers and proof readers, whose observations enriched this report.

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